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RESEARCH ARTICLE

Acquisition and expression of memories of distance and direction in navigating wood ants

A. Sofia D. Fernandes^{1,2}, Andrew Philippides^{2,3}, Tom S. Collett^{1,*} and Jeremy E. Niven^{1,2,*}

ABSTRACT

Wood ants, like other central place foragers, rely on route memories to guide them to and from a reliable food source. They use visual memories of the surrounding scene and probably compass information to control their direction. Do they also remember the length of their route and do they link memories of direction and distance? To answer these questions, we trained wood ant (*Formica rufa*) foragers in a channel to perform either a single short foraging route or two foraging routes in opposite directions. By shifting the starting position of the route within the channel, but keeping the direction and distance fixed, we tried to ensure that the ants would rely upon vector memories rather than visual memories to decide when to stop. The homeward memories that the ants formed were revealed by placing fed or unfed ants directly into a channel and assessing the direction and distance that they walked without prior performance of the food-ward leg of the journey. This procedure prevented the distance and direction walked being affected by a home vector derived from path integration. Ants that were unfed walked in the feeder direction. Fed ants walked in the opposite direction for a distance related to the separation between start and feeder. Vector memories of a return route can thus be primed by the ants' feeding state and expressed even when the ants have not performed the food-ward route. Tests on ants that have acquired two routes indicate that memories of the direction and distance of the return routes are linked, suggesting that they may be encoded by a common neural population within the ant brain.

KEY WORDS: Visual navigation, Vision, Path integration, Odometry, *Formica rufa*

INTRODUCTION

One attraction of researching insect navigation is the opportunity that it offers to understand memory processes in an animal with a brain that is considerably smaller than that of most vertebrates. Consider a desert ant, such as *Cataglyphis fortis*, that forages individually (Wehner et al., 1983). It leaves its nest to explore the surrounding and initially unfamiliar terrain for prey items. After a while, whether or not it has found food, the ant must return to the nest, if only to cool down. The ant is then able to take a rapid and direct path over unfamiliar ground to its nest because it possesses a navigational mechanism known as path integration (PI) (Wehner and Srinivasan, 2003). With the aid of PI, on its outward journey the ant combines the distances and directions that it travels into a vector

of its current distance and direction from the nest. The value of this 'home vector' can be remembered, at least for a short period (Ziegler and Wehner, 1997; Cheng et al., 2006; Narendra et al., 2007). Once an ant has expressed its home vector and reached its nest, its current PI state has dropped to zero, but it still retains a longer-term memory of its PI state at the feeding site. This memory later helps generate a 'food vector', enabling the ant to return directly to the foraging site (Bolek et al., 2012; Collett et al., 1999; Schmid-Hempel, 1984; Wehner et al., 2004).

Path integration is of limited precision and as a foraging ant repeats a PI-derived path to and from a reliable foraging location, it rapidly becomes familiar with the landscape and forms long-term view-based memories that both specify the exact route (Collett, 2010; Collett et al., 1992; Cornetz, 1910; Graham et al., 2003; Harris et al., 2007; Narendra et al., 2013) and act as an attractor, guiding ants to a familiar goal (Åkesson and Wehner, 2002; Graham et al., 2004; Wystrach et al., 2012). Ants can also form vector memories of route segments, instead of the whole route from feeder to the nest (*C. fortis*: Collett and Collett, 2009; Collett et al., 1998; Knaden et al., 2006; *Apis mellifera*: Collett et al., 2002; Srinivasan et al., 1997). An ant's path is thus controlled by a combination of its vector memories, its visual memories and a continuously updated PI state (Collett, 2012).

Although evidence for PI-derived vector memories on food-ward routes is secure, the evidence for vector memories on homeward routes is more uncertain because of likely interactions between them and an ant's PI state (Collett, 2012; Collett and Collett, 2015). These interactions can occur both if the ant is tested just before it enters its nest, when vector memories and PI will act in opposite directions, and if the ant is taken at the feeder, in which case the two tend to be in similar directions. Here, we compare the acquisition and expression of food-ward and homeward memories of ants that are in the same PI state as they are when at the start of a food-ward vector. To put ants in this state, they are taken straight from the nest, where their PI state seems to be reset to zero (Knaden and Wehner, 2006), and then they are either fed immediately before being tested or tested without being fed. Unfed ants should exhibit a food-ward vector, whereas fed ants should be motivated to return to the nest and there offload the food that they have collected. The fed ants have the opportunity to perform a remembered homeward vector with possibly less interference from their PI state than if they were tested when they had either reached the feeder or almost completed their homeward journey (Dyer et al., 2002; Harris et al., 2005).

To reduce possible masking of vector memories by ants learning the position of a goal through visual cues, we performed these experiments in a channel that restricted the ant's view of the surroundings. In addition, we shifted the start- and endpoints of the route between each training trial whilst keeping the length of the route constant so that the endpoint could not be specified visually. While these precautions reduce the likelihood that distance is determined by landmark cues, they leave open the question of

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whether the vector memories of wood ants in these experiments encode directional information from a sun compass, the major cue used by *C. fortis* during PI, or whether they obtain directional cues from the visual panorama, as wood ants do in the laboratory (Harris et al., 2007), or whether both types of cue are recorded (Towne and Moscrip, 2008). Evidence that ants can store the compass direction of a homeward route, or of a route segment, comes from a study on *Gigantiops destructor* (Beugnon et al., 2005). Individual *G. destructor* foragers hunt for termites in familiar sites in the rainforest. When ants are taken from their nest and displaced either to their familiar foraging site or to other visually unfamiliar sites and have found a termite, they immediately head for a short distance in their familiar homeward compass direction.

Through these experiments we tackled three main questions. First, do wood ants acquire and use vector memories of a homeward route? Second, if they do acquire such vector memories under what circumstances are they acquired? Do they, for instance, also learn a longer-term vector memory of the homeward journey through repetition of an outward route, when they are not allowed to perform a homeward route? Third, are memories of direction and distance independent or linked?

MATERIALS AND METHODS

Animals

A wood ant (*Formica rufa* Linnaeus 1761) colony was collected from Broadstone Warren, East Sussex, UK (Latitude: 51.077813; Longitude: 0.028814) and housed in a large plastic container (0.4×0.8×1.4 m) from June to September. Part of the time the container was outdoors under a shelter and part of the time it was in a greenhouse. The colony was supplied with sucrose solution, water and frozen crickets. Prior to and during each experiment the amount of sucrose solution was reduced.

Each experiment began by selecting more than 100 ants from the colony. These ants were placed at one end of a 9 m channel. A glass slide with sucrose solution was placed at the opposite end of the channel. Those ants that walked to the food and fed were transferred to a second channel parallel to the first one. Approximately 40 of those ants that walked back in the direction of the original release site were selected for further training. These ants were marked individually with spots of coloured paint on their thorax and abdomen.

Experimental apparatus

Two channels (9×0.07 m) closed at either end were constructed from white plastic guttering (B&Q, Southampton, UK) and placed parallel to one another separated by approximately 20 cm (Fig. 1). The channels were aligned from east to west in a greenhouse. The inner sides of each channel were painted with Fluon (AGC Chemicals Europe Ltd, Lancashire, UK) to prevent ants climbing them, and the bottom of each channel was lined with paper sheets (0.07×0.42 m). Ants were allowed to run the whole length of both channels over these paper sheets, prior to any experiment. The paper sheets were then shifted or turned randomly during training and testing to ensure that pheromone trails deposited by ants during training could not act as a guide for subsequent ants.

Training

Marked ants were selected from the colony and their identity noted. Groups of six ants were placed into a pot (7 cm) within the channel with a small opening at the base facing the feeder's direction. Ants entered the channel through this opening and walked along the channel until they encountered a glass well slide on which sucrose solution was placed. Ants tend to walk towards tall, dark objects (Santschi, 1913), so to encourage the ants to approach the feeder, particularly when they were trained to forage in both directions, a large black cylinder (45×15 cm) was placed above the channel 10 cm behind the position of the glass slide and sucrose (Fig. 1). The direction and distance between the starting pot and the feeder were kept constant within experiments, but pot, feeder and cylinder were moved together along the channel between trials so ants could not rely on visual cues from the greenhouse to locate the feeding site.

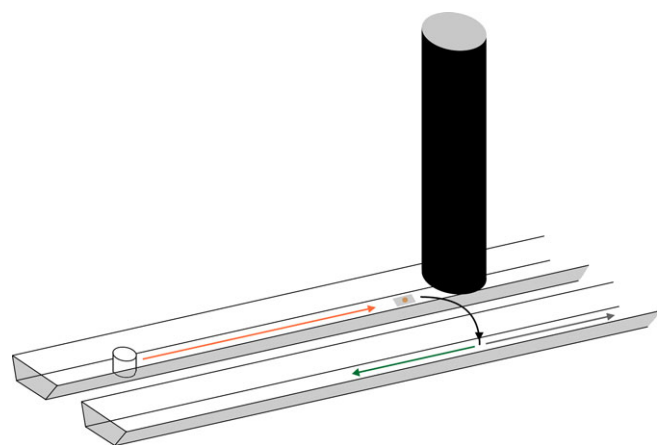


Fig. 1. Diagram of the training apparatus. Two plastic channels (9×0.07 m) were placed parallel running East–West. During training, the ants were placed into a pot (small cylinder) within one channel. Ants entered the channel through a small opening at the base of the pot facing the feeder, and walked along the channel (orange arrow) until they encountered a glass slide on which sucrose solution was placed (small grey rectangle with orange circle). On some training trials, a black cylinder (45×15 cm) was placed above the channel 10 cm behind the position of the slide/sucrose. Upon reaching the sucrose, the ants were transferred on the slide to the adjacent channel (black arrow). Once the ants had finished feeding they walked away from the slide/sucrose either in the homeward direction (dark green arrow) or in the opposite direction (grey arrow).

The ants experienced one of several training regimes. Training differed in the distance and direction of the feeder from the starting pot and whether or not ants were allowed to perform the return leg of their journey. During unidirectional training with a return route, ants travelled 2 m, always in the same direction, to find a glass microscope slide with sucrose. Once an ant had reached the slide and had begun feeding, we transferred ant and slide to the adjacent channel, without rotation. After ants had finished feeding, they tended to walk away from the sucrose. Once the ants had started to walk, we recorded the direction in which they walked. As ants often moved back and forth close to the slide before settling on a direction, we took as a criterion of their having chosen a direction that they had walked at least 50 cm away from the slide. We then recorded the first position at which they turned around and walked more than 10 cm towards the slide. After ants had turned or reached the end of the channel, we returned them to the colony. Some ants remained on or near the slide for many minutes after they had stopped feeding. These ants were also returned to the colony. Two cohorts were trained; for one the feeder was always west of the starting point and for the other the feeder was to the east.

Two additional unidirectional experiments were performed, in which ants were not allowed to perform a return route. In the first one, ants were trained on the outward route as before, but, as soon as ants had finished feeding, they were returned to the nest. The second experiment without a homeward route was conducted in the same way but the large black cylinder was absent. In both experiments, two cohorts were trained: one had the feeder east of the start and the other with the feeder to the west.

In the two experiments in which ants were trained to forage in both directions (bidirectional training), we switched the direction of the sucrose reward from the starting pot so that on alternate trials, the feeder was either east or west of the starting pot. In one experiment, the feeder was placed 3 m from the start for both training directions. In the other experiment, the feeder was 2 m away when the feeder was to the east of the start and 4 m when the feeder was to the west. In both experiments, ants were transferred to the second channel while feeding to perform the return leg of their journey. The direction and distance that ants travelled after leaving the feeder were recorded as described above. Irrespective of their direction of travel in the return channel, ants were replaced in the nest once they had turned or reached the end of the channel. We aimed to give ants 6 training trials each day, but the precise number of trials per individual varied because not all the

marked ants were found on the surface of the nest on every training trial. Because memory consolidation in insects is likely to occur overnight during sleep (*Drosophila melanogaster*: Li, et al., 2009; Joiner et al., 2006), as it does in other animals (humans, mammals and birds: reviewed in Walker and Stickgold, 2004), we pooled the results of the trials over each day when showing the time-course of learning.

Behavioural testing

At several points during each set of training trials, individual ants were tested for their ability to move appropriately according to their feeding state. Tests were given towards the end of each day's training. On 'unfed' tests, individual ants taken from the nest were placed in the channel in a 5-cm-diameter cylindrical pot with a single entrance that faced the channel wall. The direction in which the ant walked after leaving the pot was recorded. We used the same 50 cm criterion as in training to decide upon the ant's direction of travel. On 'fed' tests, individual ants taken from the nest were placed singly in a box with a feeder slide. Once the ant had begun to feed, the slide and ant were placed in the channel. Once the ant moved after feeding, its direction and distance of travel were recorded using the criteria described above.

Statistical analysis

Because of variability in the number of ants per trial and the low number of ants in some of the experiments, the analysis was conducted by pooling trials across ants to give the 'number of training trials' or the 'number of test trials'. The number of training trials in the food-ward and homeward directions during training or testing was tested for deviation from a null hypothesis that assumes no directional preference ($P=0.5$) using a two-tailed exact binomial test. We used a G -test of independence to compare between different training regimes the proportions of training trials in which ants walked in a particular direction (Sokal and Rohlf, 2012). In all cases, the G -statistic was adjusted using William's correction to account for small sample sizes. A Wilcoxon rank-sum test was used to compare the median distances walked under different training regimes. All statistical analyses were performed using programs from R (R Studio 0.97, R Studio, Inc.).

RESULTS

Unidirectional training

We trained marked ants to follow a short foraging route in a greenhouse. Ants were taken from their colony and placed in a 9 m channel running East–West. The channel contained a feeder (a drop of sucrose solution on a microscope slide) placed in front of a black cylinder (Fig. 1). The ants walked 2 m to the feeder from a cylindrical starting pot with a single exit facing the feeder and cylinder. The direction and distance between the pot, feeder and cylinder were kept constant, but the position of the route within the channel was varied to prevent the ants learning the route relative to any objects visible from the channel. Once an ant began to feed, both slide and ant were transferred to a second channel parallel to the first, allowing the ant to travel in either direction or remain at the feeder without interacting with ants approaching the feeder along their 'food-ward' paths (Fig. 1). The direction and usually the distance of this 'return' path were recorded on every trial.

The experiment was performed with two separate cohorts. The first was trained with food to the West of the starting pot, the second with food to the East (Fig. 2). Most ants left the pot and walked in the direction of the feeder and cylinder. When transferred to the parallel channel, these ants travelled mainly in the direction opposite to the food-ward path (Fig. 2) – the 'homeward' direction (247/353, $P<0.001$; exact binomial test; pooled data from both cohorts). We measured the distance that the ants travelled from the feeder to the first point that they turned in the channel (Fig. 2). Pooling these distances across both cohorts, and ignoring the paths of ants that reached the end of the channel without turning ($N=20$), gave a median path length of 2.4 ± 0.197 m [$N=227$, 95% confidence interval (CI)].

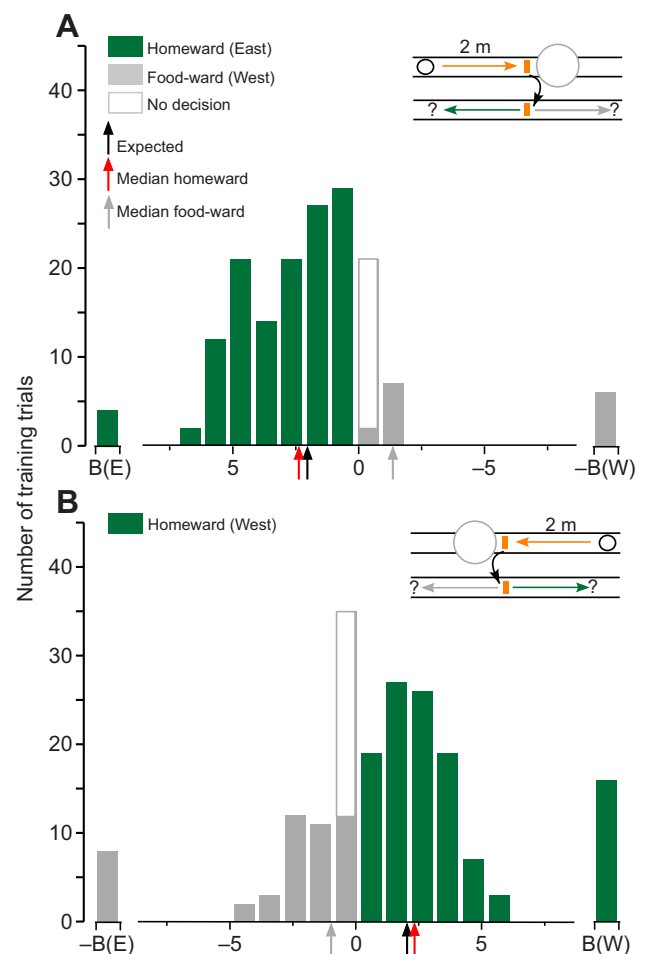


Fig. 2. Directions and distances walked during unidirectional training of wood ants (*Formica rufa*). Distribution of the distances travelled by ants in the test channel during unidirectional training with the feeder (A) 2 m West or (B) 2 m East of the starting pot. All trials were pooled. Negative values (grey) indicate ants that walked away from home (food-ward) after being transferred to the test channel. Positive values (green) show ants that walked in the homeward direction. Here, and in subsequent histograms, the bin -1 to 0 includes ants that did not move away from the feeder (white box, grey outline), 'B' and '–B' indicate ants that travelled the whole length of the channel until the end without turning back, a black arrow indicates the distance the ants would be expected to walk in the homeward direction based upon the distance of the food-ward journey, a red arrow indicates the median of the distribution on the homeward direction and a grey arrow indicates the median of the distribution on the food-ward direction. In this and subsequent figures, inset shows the training or testing procedure implemented.

Is the return path of ants trained in this way guided by path integration (PI)? To eliminate any possible contribution from route memories, we focussed on the direction of the ants' very first return before any memories of the return path could have been formed. When transferred to the parallel channel, the majority of ants moved in the homeward direction (37/52, $P<0.005$; exact binomial test), and continued to do so on subsequent trials (Fig. 3). On the first trial, these ants travelled a median distance close to the expected 2 m (1.95 ± 0.47 m, $N=35$, 95% CI). Given that the ants had no other information with which to guide their homeward route, this behaviour suggests that, at least on the first trial, the ants use PI to do so.

Our next question was whether route memories also play a role in guiding the ants' food-ward and homeward paths. To answer this question, we took ants directly from the nest and placed them in the channel, either in an unfed or a fed state. In so-called 'unfed' tests,

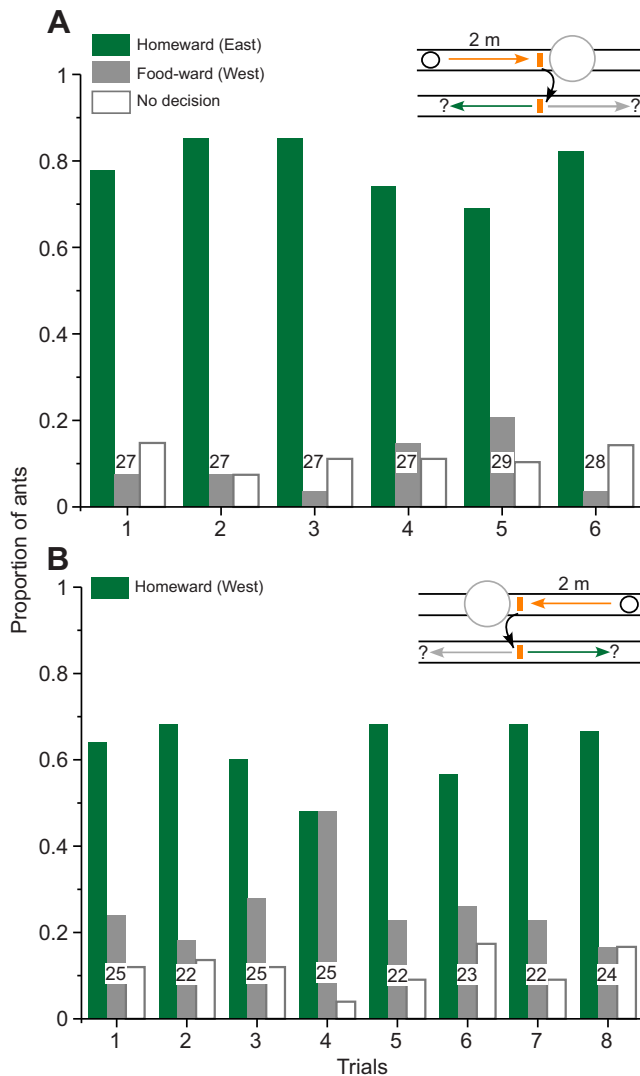


Fig. 3. Directions walked after feeding of wood ants during successive trials of unidirectional training. The directions in which ants travelled on each trial, with the feeder (A) 2 m West or (B) 2 m East of the starting pot. The green colour represents ants that walked away from the feeder in the correct homeward direction. Grey represents the ants that travelled in the wrong direction (food-ward) after feeding. Ants that stayed at the feeder are shown in white (grey outline). The numbers shown on the bars correspond to the number of ants in each trial.

ants taken from the nest were placed in a starting pot with an exit facing the side of the channel. In these tests, the black cylinder indicating the direction of the food was absent. We recorded the direction in which the ants travelled. In ‘fed’ tests, ants were taken from the nest, placed in a box containing a slide with sucrose solution, and transferred to a channel on the slide once feeding began. Again, the black cylinder indicating the direction of the food was absent. We recorded the direction and distance that ants walked after leaving the slide (Fig. 4). Ants in both tests often walked back and forth a short distance before selecting a direction of travel (see Materials and methods).

In unfed tests, the majority of ants chose the food-ward direction (21/24, $P < 0.001$; exact binomial test). In fed tests, ants consistently chose the homeward direction (49/69, $P < 0.001$; exact binomial test), travelling a median distance of 1.48 ± 0.47 m ($N = 46$) before turning (Fig. 4). Because ants in the two tests travelled appropriately

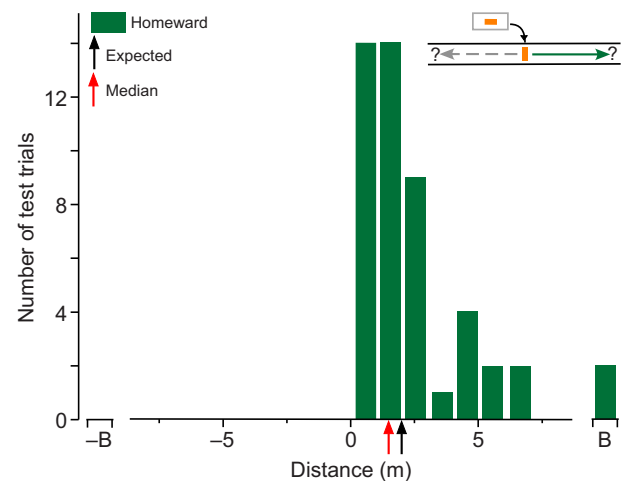


Fig. 4. Directions and distances walked by wood ants during fed tests following unidirectional training. The distances walked in the homeward direction for all the fed tests pooled (green). The median of the distribution for all the fed tests is indicated by the red arrow and the expected distance by the black arrow.

in a food-ward or homeward direction despite being in the same PI state, we suggest that being in a fed state was sufficient to prime the ants to walk in the homeward direction guided by vector memories of the return path.

Unidirectional training omitting homeward training

Ants may form an explicit memory of their return path or, alternatively, feeding may cause them to invert their remembered food-ward route. To distinguish between these possibilities, we trained ants to walk from the starting pot to the feeder, as before, but returned them to the nest immediately after feeding, thereby preventing them from performing their homeward route. This experiment was carried out in two ways with two cohorts trained for each variant to ensure that the ants behaved similarly, whether the feeder was east or west of the starting pot. In the first variant, the cylinder was present during training and absent during tests, whereas in the second variant, there was no cylinder in training or in tests.

With the cylinder present in training, the proportion of ants in fed tests travelling in the homeward direction was significantly less when they had been prevented from performing the homeward path during training compared with ants that had been allowed to do so ($P < 0.005$; G -test) (Fig. 5Ai,ii). In contrast, on unfed tests, the majority of ants still moved in the food-ward direction (47/75, $P < 0.05$; exact binomial test) (Fig. 5Bi,ii). Without the cylinder present, the proportion of ants in fed tests travelling homeward was again small and did not differ significantly from those trained with the cylinder present during training ($P > 0.1$; G -test) (Fig. 5Aii,iii). Unfed ants walked mostly in the food-ward direction (42/47, $P < 0.001$; exact binomial test) (Fig. 5Bi–iii). It thus seems that to express their homeward memories, ants need to practise the return path, whether or not the visual surroundings are the same or different during training and testing. Furthermore, these experiments suggest that after feeding, ants are only primed to travel in the homeward direction if they have formed memories of their homeward route.

Bidirectional training

Bidirectional training, in which an ant learns two foraging routes in opposing directions along the channel, allowed us to study the roles of PI and vector memories in controlling the ants’ choice of

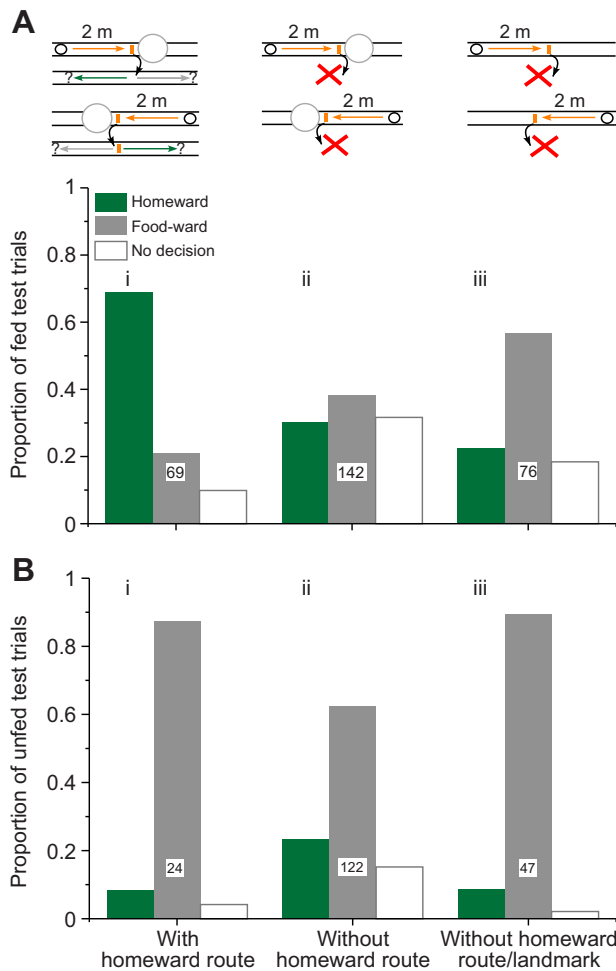


Fig. 5. Directional decisions of wood ants during fed and unfed tests after unidirectional training. The proportion of (A) fed or (B) unfed test trials in which an ant walked homeward (green), food-ward (grey) or did not walk at all (white columns, grey outline) after unidirectional training during which ants were (i) allowed to perform the return journey; (ii) returned to the nest immediately after feeding; (iii) returned to the nest immediately after feeding in the absence of the black cylinder. The numbers shown in the bars are the number of ants in each training regime.

homeward direction. This training also permitted us to examine whether there is a link between the distance and the direction that an ant travels on the two different return routes. An ant with knowledge of two such foraging routes must use information from its food-ward trip to determine the correct homeward direction. At the start of training, on its very first trip in a homeward direction, the choice of direction is probably informed and controlled entirely by PI, as is likely to be the case with unidirectional training. On subsequent homeward trips, the chosen distance and direction may be controlled solely by PI, or by vector memories primed by the ant's PI state and/or by a signal related to the expression of memories of the food-ward route.

To make ants learn two foraging routes, we modified the training paradigm by alternating the feeder's direction from the starting pot between westward and eastward on each trial. We performed two bidirectional experiments: in the first, the distance between the starting pot and the feeder was 3 m in both training directions, and in the second, the path length was 2 m westward and 4 m eastward.

As a first step in studying the control of the ants' homeward direction, we plotted the ants' choice of homeward direction after

feeding on successive trials (Fig. 6). On the first trial of the 2/4 m training there was a preference for the correct direction (7/9 ants) as was the case during unidirectional training, so giving evidence of control by PI. Oddly, there was no similar preference on the first trial of the 3 m training (4/12 ants). After the first trial, both training regimes exhibited an improvement over time (Fig. 6), that was absent in unidirectional training (Fig. 3). A period of confusion after the first trial suggests that processes, possibly the formation and incorrect priming of memories of the return path, are interfering with appropriate behaviour. Once memories are expressed correctly and can reinforce or substitute for the control by PI, correct choices are restored.

A progressive improvement in the ants' correct choice of direction and distance occurred across the first 2 or 3 days (Fig. 6). In both bidirectional experiments, the proportion of ants travelling in the homeward direction increased after the first day of training. The proportion of ants travelling in the homeward direction was significantly greater than chance on the second to the fifth day of training for both the 3 m and 2/4 m training (3 m, 184/246, $P < 0.001$; 2/4 m, 296/391, $P < 0.001$; exact binomial tests). The proportions of ants travelling in the homeward direction from the second to the fifth day were significantly greater than chance irrespective of whether the feeder was eastward (3 m, 97/118, $P < 0.001$; 2/4 m, 147/193, $P < 0.001$) or westward (3 m, 91/128, $P < 0.001$; 2/4 m, 149/198, $P < 0.001$).

A similar improvement in accuracy was seen in the distance travelled on return paths. During 3 m training, the median distance of the ants' return paths was close to 2 m on the first day of training (2.1 ± 0.7 m, $N = 33$) but increased significantly in some of the following days (Fig. 6Aiii). Pooling together the second to the fifth day, the median distance travelled by the ants on the homeward direction was close to 3 m (2.9 ± 0.2 m, $N = 175$). During 2/4 m training, the median homeward distance also started off close to 2 m (4 m direction, 2 ± 0.8 m, $N = 17$; 2 m direction, 2.5 ± 0.97 m, $N = 15$) on the first day. However, on every following day, the median distance travelled on the 4 m trial was significantly higher than on the 2 m trial (Fig. 6Biii). Pooling together the second to the fifth day, the median distance walked homeward on 4 m trials was 4.25 ± 0.31 m ($N = 128$) and 2.05 ± 0.24 m ($N = 130$) on 2 m trials.

In sum, after the first day of bidirectional training, the distances travelled by the ants on their return paths were broadly distributed, but biased towards the distance of the food-ward journey (Fig. 7). The homeward distances during the westward trials of the 3 m training (2.6 ± 0.33 m, $N = 83$) were significantly greater than for the 2 m westward trials of the 2/4 m experiment (2.05 ± 0.24 m, $N = 130$) ($P < 0.05$; Wilcoxon test). Similarly, for eastward trials, the distance covered in the 3 m training was significantly smaller than that covered in the 4 m training (3.08 ± 0.31 m, $N = 92$; 4.25 ± 0.31 m, $N = 128$, $P < 0.01$; Wilcoxon test). The improvement across days in the choice of direction and in the distance travelled on return paths suggests that the control of the return path is likely to involve both properly expressed vector memories and PI.

So far we have considered group performance and neglected individual variation. How accurately do individual ants express the directions and distances of their return paths? The percentage of trials on which ants choose the correct direction on eastward and westward food-ward trials varied widely between individuals. However, the level of performance tended to be consistent for directional choices in the two directions: ants that make correct or incorrect choices on eastward trials tended to be similarly good or bad on westward trials (Fig. 8A,B). To assess whether individual ants are precise in their estimate of distance, we took ants that chose

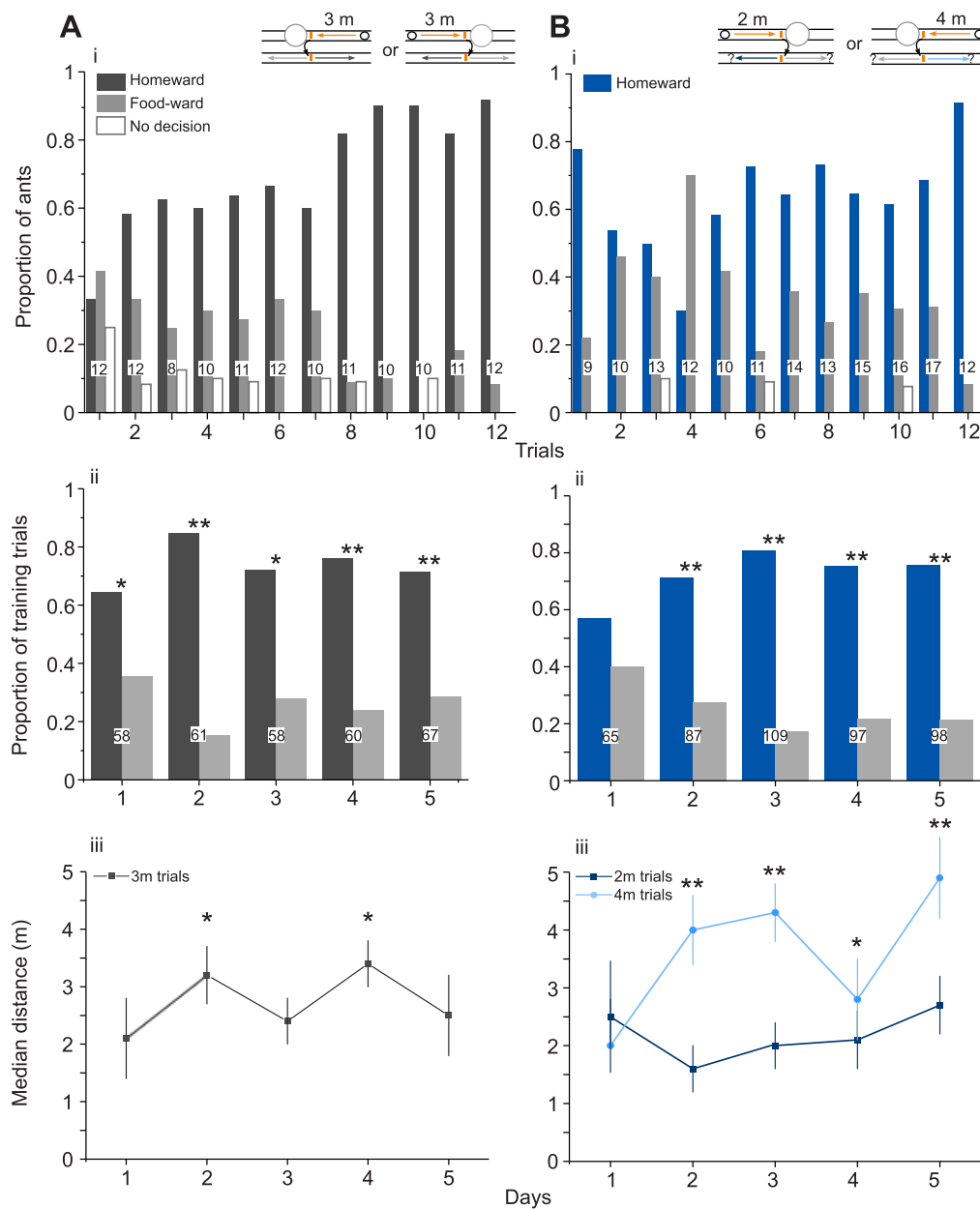


Fig. 6. Performance of wood ants across days during bidirectional training. (A) The position of the feeder alternated between 3 m West (odd numbers) of the starting pot and 3 m East (even numbers). (B) The position of the feeder alternated between 2 m West (odd) or 4 m East (even) away from the starting pot. (i) The directions ants travelled on the first 12 trials (2 days) of bidirectional training. Ants that stayed at the feeder are represented by the white columns (grey outline). The numbers shown in the bars indicate the total number of ants that reached the feeder on each trial. (ii) The proportion of training trials on each day on which ants travelled in the homeward or food-ward direction after feeding. Asterisks indicates that the proportion of training trials in which ants travelled in the homeward direction was significantly (* $P < 0.05$, ** $P < 0.01$, exact binomial test) greater than chance. The numbers shown in the bars correspond to the number of training trials on each day. (iii) The median distance travelled by ants after feeding on those training trials in which ants walked in the homeward direction. The data shown for each day pools distances irrespective of the direction of the feeder. Asterisks in Aiii on the second and fourth days indicate significantly longer distances than the first day. Asterisks in Biii indicate days in which the distances walked in 4 m trials were significantly greater than during 2 m trials. * $P < 0.05$, ** $P < 0.01$; Wilcoxon test.

the correct direction on at least 70% of trials in each direction and plotted the mean distance that each ant travelled when it chose the correct direction on eastward training trials against the equivalent distance on its westward training trials (Fig. 8C,D). The distances of the westward or eastward homeward paths travelled by individual ants during 3 m training were similar, lying close to the expected $y=x$ line. However, the mean distance of the homeward paths of most individual ants was greater on 4 m eastwards trials than on 2 m westwards trials, straddling the expected $y=2x$ and lying above the $y=x$ line. When these individual mean distances are turned into ratios by dividing the eastward distance by the westward distance, the ratios of individuals trained with 3 m distances were significantly smaller than were those of individuals trained to 4 and 2 m distances ($P < 0.01$; Wilcoxon test).

A tendency to link direction and distance is also apparent in fed tests performed on ants given the 2/4 m training. There is no reason to suppose that ants in fed tests will select one direction in preference to the other, but an associated memory of distance and

direction predicts that the distance travelled in a test will be related to the direction that an ant selects, as was indeed the case. The distances that the ants travelled were significantly shorter when they chose to move eastwards as in a 2 m trial (1.2 ± 0.5 m, $N=10$) than when they moved westwards, as in a 4 m trial (3.2 ± 0.7 m, $N=17$) ($P < 0.01$; Wilcoxon test) (Fig. 9). We conclude that feeding primes route memories in which direction and distance are linked.

DISCUSSION

The experiments that we have described examined the vector memories of wood ants. To promote the use of odometric rather than visual cues to control stopping, we trained ants to learn a short foraging route of a fixed length within a longer channel, shifting the starting position between trials to avoid a visual memory of the endpoint being formed. The ants performed either a single foraging route (unidirectional training) or two foraging routes in opposite directions (bidirectional training). Although our training method makes it unlikely that visual memories learnt in channels are the

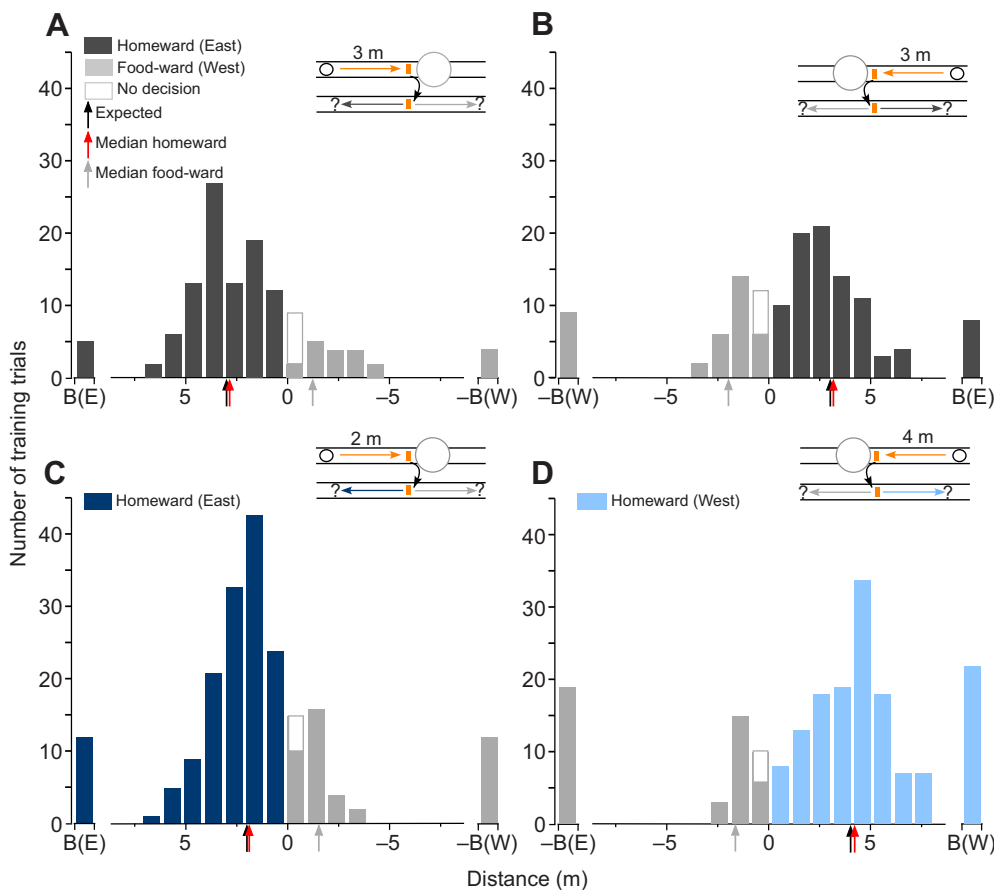


Fig. 7. Directions and distances walked by wood ants during bidirectional training. Distribution of the distances travelled in the test channel during bidirectional training with the feeder (A) 3 m West, (B) 3 m East, (C) 2 m West or (D) 4 m East of the starting pot. Negative values (grey) indicate trials in which ants walked away from home or did not move from the slide (white in bin -1 to 0) after transfer to the test channel. Positive values show trials in which ants walked in the homeward direction. Black arrows indicate the distance that ants would be expected to walk in the homeward direction based upon the distance of the food-ward journey. Red arrows indicate the median of the distribution on the homeward direction and grey arrows indicate the median of the distribution on the food-ward direction. Data are pooled across all days with the first day excluded.

major influence in setting the ants' travel distance, they may well contribute to inaccuracies in performance (Bolek and Wolf, 2015). However, views seen in the channel, as well as compass cues, are very likely to contribute to the ants' choice of direction.

A pervasive problem in interpreting these experiments is how to disentangle the contributions of the ant's current PI state from route memories derived from PI to the control of the ants' behaviour. The parameters of the ants' food-ward route are presumably set by a vector memory, as is the case in the desert ant *C. fortis* (Bolek et al., 2012; Collett et al., 1999; Schmid-Hempel, 1984; Wehner et al., 2004). But both current PI state and vector memories could in principle contribute to the homeward route because at the feeder the ants' PI state encodes the distance and direction of home. In our experiments, direct evidence that ants formed vector memories of the distance and direction of the homeward leg of their foraging route came from fed and unfed tests in which their current PI state could not have played a part. Unidirectionally trained ants, when taken from the nest, fed and placed in the channel walked in the direction of the return route for roughly the expected distance. In contrast, unfed ants walked in the direction of the feeder. The marked difference in the ant's behaviour on fed and unfed tests demonstrates that wood ants do acquire vector memories of their return journey and that these memories are expressed when ants are fed and are then in an appropriate goal state for returning home (see Dyer et al., 2002; Harris et al., 2005 for earlier evidence of motivational priming of the direction of PI vectors and of visual memories).

Expression of vector memories

The ants' behaviour during fed tests is consistent with the supposition that they express vector memories on their normal

return journey. The possibility that, in these experiments, the return route during training is informed entirely by the ants' PI state is made unlikely by the ants' behaviour on the first day of 2/4 m bidirectional training. On the very first trial, the ants move predominantly in the homeward direction after they have fed, which is consistent with guidance by PI before the formation of a homeward vector memory. But on subsequent trials, the ants move in both directions after feeding as though imperfect memories are interfering with control by PI. Only by the second day of training do ants again move consistently in the homeward direction. This improvement during training suggests that the vector memories displayed during Fed tests are also expressed during training after the ants have fed and are choosing their return path. If they were not, and the return route was guided entirely by the current PI state, there would be no reason for the accuracy of the return path to improve with increased training.

Ants trained to one route have just one homeward vector memory that is primed by feeding. Ants trained with two routes seem to choose appropriately between two possible homeward memories. Their confusion early in bidirectional training may, in part, be a consequence of having to learn which of the memories primed by feeding should be expressed. There are two sources of information that could inform an ant's decision: its PI state and the particular food-ward memory that was engaged. Although our current data do not tell us which of these sources are involved, PI state seems the more plausible. Signals from an activated food-ward memory would need to persist throughout feeding, perhaps minutes after the food-ward memory had been expressed, whereas PI state is known to persist (Ziegler and Wehner, 1997; Cheng et al., 2006; Narendra et al., 2007).

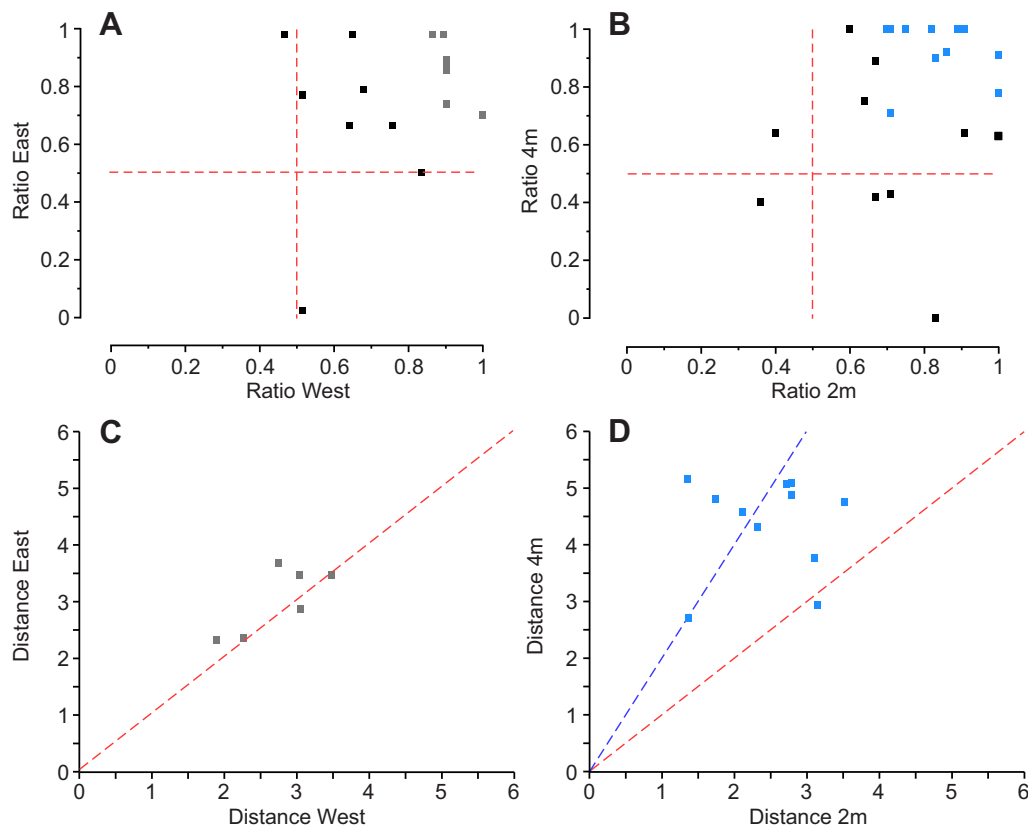


Fig. 8. Performance of individual wood ants during bidirectional training. (A) The ratio of the number of trials with the feeder 3 m West of the starting pot in which an individual ant walked in the correct direction to the total number of trials is plotted against the same ratio for that ant calculated when the feeder was 3 m East. The majority of ants, represented by points in the upper right hand quadrant of the graph, walked in the correct direction in both directions. Those ants that were correct on 70% or more of trials are indicated in grey. Data are shown for 16 individuals. (B) Feeder was placed 2 m West versus 4 m East. Those ants that were correct on 70% or more of trials are indicated in blue. Data are shown for 22 individuals. (C) The median distances on correct trials travelled by individual ants in each direction trials with the feeder 3 m West of the starting pot versus the median distances travelled with the feeder 3 m East. The red dashed line indicates equal distances walked. Data are shown for 6 individuals. (D) The feeder was placed 2 m West versus 4 m East. The blue dashed line indicates the expected distances, if ants were to walk twice as far on their 4 m homeward route than on their 2 m one. Data are shown for 12 individuals.

Acquisition of vector memories of the return path

During unidirectional training, most ants walked directly towards the feeder upon leaving the starting pot. The configuration of the route, consisting of a starting pot with a single exit in the direction of

the feeder, a narrow channel with one or more microscope slides at the end of the route and a large black cylinder, could be largely responsible for the appropriately directed movement. That ants acquired memories of the food-ward direction was shown by the results of unfed tests early in training. After only 5 trials, the majority of the ants walked in the food-ward direction. In these unfed tests, the cylinder indicating the position of the food within the channel was absent, suggesting that this prominent visual landmark is unnecessary for ants to determine the food-ward direction.

In unidirectional training, the rapid acquisition of the food-ward direction is paralleled by the rapid acquisition of the return route, as shown by fed tests that can be successful after only 4 training trials (data not shown). In these tests, the initial state of the path integrator is zero, suggesting that the ants have acquired a memory of the return route. This memory is acquired irrespective of whether an object is placed next to the food or not. One can imagine two rather different ways in which vector memories of a homeward route are formed. The first possibility is analogous to storing a home vector in longer-term memory; the memory is formed automatically when a home vector is computed at a significant location, like a feeder. Our experiments suggest that this possibility is unlikely. When ants were trained by being picked up from the feeder and given no opportunity to perform a homeward route, the results of Fed tests were abnormal. Instead of moving consistently in the homeward direction, ants

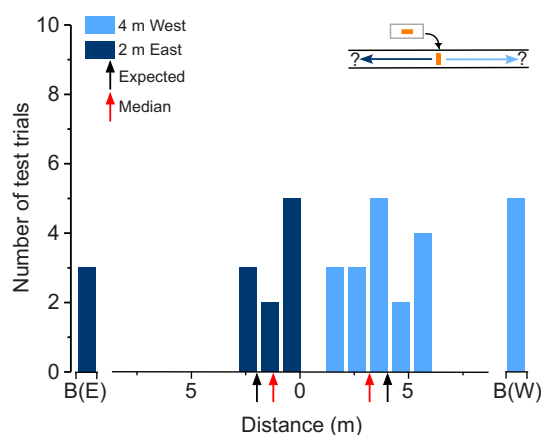


Fig. 9. The distances walked by wood ants in fed tests following bidirectional training. The distance travelled in fed tests trials by ants trained with the feeder alternately 2 m West and 4 m East of the starting pot. Distances walked in the 2 m direction (East) are negative and those in the 4 m direction (West) are positive.

sometimes moved consistently in the food-ward direction. Sometimes they moved in the food-ward direction as often as in the homeward direction and sometimes they did not move at all. It seems that vector memories of the homeward distance and direction are formed while performing routes. This active mode of route acquisition is suited to learning routes through uneven or cluttered terrain, when direction will vary along a segment, although we cannot be sure whether ants treat the routes in the channels as a complete foraging route or as segments of longer routes.

Do odometric memories link direction and distance?

Fed tests on ants with unidirectional training indicate that ants acquire memories of distance as well as of direction, but as training was always with the same 2 m distance, no comparison of different distances can be made. Evidence that ants do store the distance of the return path and also link direction and distance comes from fed tests on ants with 2/4 m bi-directional training. In these tests, the ants' responses are independent of their PI state. With homeward memories in both directions and no priming of their food-ward route, ants can choose to walk in either direction. The significant finding is that when ants move in the direction consistent with their taking the 2 m route, their distance before turning is shorter than when they move in the opposite direction that they would take on the 4 m route. The distance that ants walk is thus associated with their choice of direction. This linkage between direction and distance is of particular interest because it is consistent with a plausible encoding of a distance–direction vector using a population of direction cells, with distance represented by some property of the appropriate subset of direction cells. This encoding also fits with the priming of odometric memories by a vector computed through PI.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

A.S.D.F., A.P., T.S.C. and J.E.N. conceived the project. A.S.D.F., T.S.C. and J.E.N. designed and performed the experiments and analysis. A.S.D.F., T.S.C. and J.E.N. wrote the paper with input from A.P.

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